Microbial biomass and community structures in the burrows of bromophenol producing and non-producing marine worms and surrounding sediments

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ABSTRACT: Microbial biomass and community structures were determined in sediments lining the burrows of 3 species of marine worms, and in nearby surface and subsurface sediments, using ester-linked phospholipid fatty acid (PLFA) analysis. The potential impact of biogenic bromophenols produced by 2 of these animals on burrow microbial communities was of particular interest. The burrow microbial communities were markedly different from those of surrounding surface and subsurface sediments. Differences in microbial biomass were attributed to burrow structures, textures of the burrow lining sediments, and organic carbon content. No significant reduction of microbial biomass or of several distinctive signature PLFA was detected in bromophenol-contaminated burrows, when compared to non-bromophenol-containing burrows. All 3 types of sediments (burrow, surface, and subsurface) examined for each worm species were distinct as determined by multivariate cluster analysis of PLFA profiles. Signature lipid biomarker PLFA for Gram-negative bacteria, Gram-positive bacteria, sulfate-reducing bacteria and anaerobes, and microeucaryotes were readily identified in burrow sediments from bromophenol producing and non-producing worms.

KEY WORDS: Microbial communities · Infaunal worms · Phospholipid fatty acids · Marine sediment

INTRODUCTION

Marine infauna modify sediments through burrow formation, burrow irrigation, defecation, locomotion, and excretion of soluble and insoluble exudates, resulting in physical and chemical alteration of the local sediment environment (Aller 1978, 1983, Beukema & de Vlas 1979, Beukema 1982, Woodin et al. 1987). One important result of the construction and irrigation of burrows is formation of radial chemoclines of oxidized and reduced solutes, analogous to the vertical discontinuities seen at the sediment-water interface (Fenchel & Reidel 1970, Aller & Yingst 1978, Aller 1980, Boudreau & Marinelli 1994). These environmental modifications significantly affect resident microbial communities, with the most pronounced impacts occurring in sediments lining the infaunal burrows (Dobbs & Guckert 1988b, Jensen et al. 1992) where steep gradients of potential electron donors and acceptors overlap (Aller 1983). The unique physical and chemical character of infaunal burrow linings is fairly stable compared to the more frequently disturbed sediment-water interface sediments, resulting in formation of microbial communities fundamentally different from those of nearby surface and subsurface sediments.

Microbial communities which line burrows are most accurately described as biofilms, complex accretions of microorganisms and exopolymers (Lappin-Scott & Costerton 1989). A variety of features of the burrow

microenvironment contribute to defining the extent and composition of the biofilms which line the burrows. Infaunal organisms frequently process the sediment in the burrow lining, altering particle size distribution (Aller 1978, 1983). Available surface area, burrow wall permeability, and fluxes of nutrients through the burrow wall are all dependent on the structure of the burrow and on the macrofaunal organism inhabiting it. The chemical environment of the burrow is also strongly affected by the macrofaunal inhabitant. Mucopolysaccharides and a wide variety of low molecular weight compounds can be produced abundantly within the burrows, presumably affecting burrow microbial communities either positively or negatively (Sheikh & Djerassi 1975, Aller 1983, Schottler et al. 1983, King 1986, Woodin et al. 1987, Jensen et al. 1992). Which of these burrow and infaunal characteristics is most important in determining the character of the burrow lining biofilm is presently unknown.

Of the many burrow physico-chemical properties and infaunal activities which may affect the formation and character of the burrow lining biofilm, exudation of soluble metabolites is of particular interest. A variety of infaunal polychaetes and hemichordates excrete halogenated secondary metabolites (Ashworth & Cormier 1967, Higa & Scheuer 1975a, b, Sheikh & Djerassi 1975, King 1986, Woodin et al. 1987, Corgiat et al. 1993). These organisms contaminate their environs with toxic and odoriferous bromoaromatics (King 1986, Woodin et al. 1987, Steward et al. 1992, D. E. Lincoln, K. T. Fielman, R. A. Marinelli & S. A. Woodin unpubl.), which reach their highest concentrations in the burrow lining (King 1986, Lincoln et al. unpubl.). Worm bromometabolites have been thought to serve as antifouling agents on the surfaces of the worms and within their burrows, restricting bacterial growth, oxygen consumption, degradation of the burrow wall mucopolysaccharides, and potentially preventing infection of wounds in the worms (Ashworth & Cormier 1967, Sheikh & Djerassi 1975, King 1986, Goerke & Weber 1991). However, previous studies have shown no significant impact of biogenic bromophenols on bacteria and microalgae in contaminated wormbed sediments (Jensen et al. 1992, Steward et al. 1992). The relative importance of these potential antimicrobial compounds, as well as other features of the burrow microenvironment, in defining burrow biofilm microbial communities could best be assessed by direct examination. Such investigations are complicated by the small amount of sample material which can be collected from a burrow, the large number of samples which must be analyzed to account for inherent variability among samples, and disturbance artifacts which may be introduced by sampling and sample incubation (Findlay et al. 1990).

One approach which overcomes most of these problems is extraction and analysis of cellular components, such as phospholipid fatty acids (PLFA). PLFA are found in all bacterial and eucaryotic membranes and provide a basis for the determination of viable microbial biomass and a definition of microbial community structures in marine sediments in situ (Perry et al. 1979, White et al. 1979, Gillan et al. 1983, Baird & White 1985, Baird et al. 1985, Guckert et al. 1985, Currie & Johns 1988, Findlay et al. 1990, Rajendran et al. 1992). Since phospholipids are not used as reserve polymers and are rapidly turned over in marine sediments (White 1983, 1988) they provide a sensitive measure of the viable microbial biomass. Analysis of membrane-bound (ester-linked) PLFA after their conversion to fatty acid methyl esters assures that only PLFA derived from viable organisms are included in the analysis. Samples for PLFA analysis can be collected and immediately frozen in the field, minimizing disturbance artifacts (Findlay et al. 1990). PLFA can also be extracted from small samples, and large sample sets can be easily and reproducibly analyzed providing a sensitive and quantitative means for studying the in situ microbial biomass and community structure. Multivariate statistical analysis of PLFA profiles allows quantitative comparisons between different sample types and sample groups. While the method does not allow complete identification or quantification of all microbial groups in complex communities (see Haack et al. 1994), it has proven very useful in the determination of microbial biomass and identification of some taxonomic and functional groups in environmental samples. PLFA-based estimates of biomass have been shown to correspond to estimates based on other measures, including direct microscopic counts (Balkwill et al. 1988, White 1988), and a number of different microbial groups contain useful indicator PLFA (Federle et al. 1986, Vestal & White 1989, Tunlid & White 1992). We have examined microbial biomass and community structure in sediments lining the burrows of 3 marine worms, comparing burrow microbial communities to those in nearby surface and subsurface sediments. Of particular interest were the impacts of biogenic bromophenols and other characteristics of the burrow lining microenvironment on microbial biomass and community structure.

MATERIALS AND METHODS

Materials. Solvents from Burdick & Jackson (Muskegon, MI, USA) were residue analysis grade (GC^2). Standards and derivatizing reagents were purchased from Supelco Inc. (Bellefonte, PA, USA), Nu Chek Prep (Elysian, MN, USA) and Pierce Chemical Co. (Rock-

ford, IL, USA). Bromophenols were obtained from Aldrich Chemical Co. (Milwaukee, WI, USA) and Sigma (St. Louis, MO, USA). The purity of each compound was evaluated using gas chromatography/mass spectroscopy (GC/MS) and pure lots used without further purification.

Study site. The North Inlet estuary (33° 20' N, 79° 10' W) is a relatively small (3200 ha), bar-built *Spartina* marsh system located near Georgetown, South Carolina, USA. Dame et al. (1986) and Pinckney & Zingmark (1993) have provided detailed descriptions of this system. The capitellid polychaete *Notomastus lobatus*, maldanid polychaete *Branchyoasychus americana*, and hemichordate *Balanoglossus aurantiacus* are abundant in North Inlet intertidal sandflats, and their burrows can be recognized on site by their characteristic fecal mounds, burrow openings, or tube structures (Ruppert & Fox 1988). All are deposit feeders; *N. lobatus* and *B. americana* are head down subsurface deposit feeders. The *B. americana* tube is straight and vertical with the head shaft opening 25 to 30 cm below the sediment surface. This maldanid prefers small particles in tube construction and is often associated with clumps of more cohesive mud within sand- and mudflats (Mangum 1964a). It appears to irrigate weakly, if at all, and does not create a well-aerated microenvironment within the tube (Mangum 1964b). The upper 6 to 10 cm of *N. lobatus* burrows are straight and vertical. Below this point, the burrow becomes helical (Powell 1977), a unique structure at this sampling site. No observations of *N. lobatus* irrigation behavior have been reported, but the light brown oxidized sediment layer lining the burrow is quite thin, likely indicating burrow water stagnation. *B. aurantiacus* builds a U-shaped burrow, excavating a shallow subsurface feeding chamber at the head end (Rupert & Fox 1988). The surface sediments overlying this chamber slump, forming a small, shallow basin into which flocculent detrital materials collect and are ingested by the worm. Burrow irrigation data are not available, but the sediments lining the burrow are light colored and appear to be highly oxidized.

Sample collection. Sediment samples for PLFA analysis were collected as matched sets, with each including 1 worm burrow sediment sample, 1 surface sediment sample, and 1 subsurface sediment sample. Subsurface samples were taken approximately 10 to 20 cm deep, near but not adjacent to worm burrows. Surface sediments were collected from approximately the top 1 cm of surface sediment near but not adjacent to the worm burrows. Sediments (approximately 1 to 2 mm thickness) were scraped from the linings of the burrows for *Notomastus lobatus* and *Balanoglossus aurantiacus* using sterile spatulas, and pieces of *Branchyoasychus americana* tubes were recovered intact.

Subsurface and surface sediments were also collected using sterile spatulas. Large macrofauna were excluded from all samples. Ten sets were collected from burrows and surrounding sediments for each worm species. In all cases, positive identification of each worm was made after exhuming an identifiable portion or intact individual. The samples were placed in sterile Whirl-pak (Nasco, Ft. Atkinson, WI, USA) bags and immediately frozen on dry ice for transport to Columbia, South Carolina. Samples were stored at −80°C pending transport to Knoxville, Tennessee, USA, for extraction and analysis. Integrated bulk wormbed sediments were collected using cut-off plastic syringes (2.6 cm diameter, 11 cm long), and burrow sediment samples were collected as described above for sediment analyses. Samples of *B. americana* tissue were collected and stored in methanol (Burdick & Jackson, Nanograde) for extraction of halogenated aromatic compounds for analysis by GC/MS.

Sediment extraction and lipid analysis. The 1 to 20 g sediment samples were extracted and PLFA recovered and derivatized for identification and quantification as described by Guckert et al. (1991). Samples (1.0 μl) were injected onto the column of an IBM gas chromatograph (GC) equipped with a 60 m Rt_x–1 (nonpolar methyl silicone) column with a 30 s splitless injection time and an injection temperature of 290°C. Phospholipid fatty acid methyl esters (PLFAME) were resolved using a temperature program of 100°C to 150°C at 10°C min⁻¹, 150°C for 1 min, 150°C to 282°C at 3°C min⁻¹, and 282°C for 5 min. Hydrogen (linear velocity 35 cm s⁻¹) was the carrier gas. Detection was by hydrogen flame ionization using a 30 ml min⁻¹ nitrogen makeup gas at a temperature of 290°C. An equal detector response was assumed for all components. Peak areas were quantified using the PE Nelson 3000 series Chromatography Data System (Revision 5.0). Determination of fatty acid structures was performed using GC/MS as described by Ringelberg et al. (1986). Bacterial fatty acid double bond position and geometry were confirmed using GC/MS analysis of the dimethyl disulfide adducts of the monounsaturated PLFAME as described in Nichols et al. (1986). Additional verification was done, as needed, using equivalent chain length analysis (Christie 1989).

Fatty acid nomenclature. Fatty acids are designated as A:BωC, where A is the total number of carbon atoms, B is the number of double bonds, and C is the position of the double bond from the aliphatic end of the molecule. Geometry of this bond is indicated 'c' for *cis* and 't' for *trans*. The prefixes 'i' and 'a' refer to iso and anteiso methyl branching respectively (Kates 1986). Mid-chain methyl branches are designated by 'me' preceded by the position of the methyl group from the acid end of the molecule. Cyclopropyl fatty acids are designated as 'cy'.

Worm halometabolite analysis. Methanol extracts from *Branchyoasychus americana*, which had not been previously screened for production of halometabolites, were filtered through 0.2 µm filters to remove particulates. Extracts were examined for halometabolites using GC/MS as described previously (Woodin et al. 1993).

Sediment grain size and carbon analysis. Burrow lining and bulk wormbed sediments were dried to constant weight at 70°C and sized through a Wentworth series of sieves, using a mechanical shaker (Tyler Industrial Products, Mentor, OH, USA) to facilitate size fractionation. Percentage silt and clay was measured as the portion of sediment with a grain size ≤63 µm. Organic carbon content of wormbed and burrow sediment was determined as ash free dry weight following combustion at 500°C in a muffle furnace.

Statistical analysis. PLFA profiles were checked for normality of distribution (variances were homogeneous), biomass data were log transformed, and PLFA mole percentage data were arcsin($\sqrt{x}$) transformed prior to statistical analysis. Analysis of PLFA data was performed using multivariate cluster analysis, principal components analysis, and analysis of variance (ANOVA), Tukey-Kramer test for Honestly Significant Difference (Tukey test), and discriminant analysis programs in commercially available software packages: Ein-sight (Infometrix, Seattle, WA, USA), Statgraphics (Statistical Graphics Corporation, Rockville, MD, USA), and SAS (SAS Institute, Cary, NC, USA). Residual plots indicated homogeneous variances. A significance level of $p \leq 0.01$ was used for all analyses following a power test which showed very large power of analyses with these data.

RESULTS AND DISCUSSION

Characteristics of sediments and worm burrows

Sediments surrounding and within burrows of the 3 species of marine worms differed substantially (Table 1). *Notomastus lobatus* lives in fine-grained muddy sands. Its burrow wall sediments were tightly compacted, cohesive, polished, heavily coated with biogenic films of mucus, and organically enriched. Looking from the burrow outward into the surrounding anoxic sediment, the transition from a thin layer of oxidized (light brown) burrow lining sediment to black anoxic sediment was very apparent. *Balanoglossus aurantiacus* lives in coarser muddy sands with lower silt and clay content than *N. lobatus* or *Branchyoasychus americana*. The sediments lining these burrows were not very cohesive, had only a light coating of mucus, and were much lower in organic carbon than the other two types of worm burrows. *B. americana* builds a highly structured tube in fine sand and muds similar to those inhabited by *N. lobatus*. A portion of the tube structure usually extends above the sediment-water interface. Sediments lining these tubes are firmer than the surrounding sediment, very cohesive, and the tube can often be removed from the sediment intact. *B. americana* tube sediments are enriched in organic carbon. The inner lining of the tube is highly polished, with mucus present. The surface sediments at all locations were fine in texture and highly oxidized, as indicated by their light brown color. Previous studies have shown sediments in these and similar locations to contain high levels of diatom biomass (Steward et al. 1992, Pinckney & Zingmark 1993). All subsurface sediments were black, indicating anoxia and chemical reduction. Organic content of all integrated surface and subsurface sediments was lower than that of burrow sediments.

Bromometabolite analysis

Bromometabolites produced by these species have been identified by gas chromatography/mass spectroscopy, either previously or for this study. *Notomastus lobatus* produces 4-bromophenol, 2,4-dibromophenol, and 2,4,6-tribromophenol (Chen et al. 1991, Steward et al. 1992). *Balanoglossus aurantiacus* produces 2,6-dibromophenol (Steward et al. 1995). This compound

Table 1. Median grain size, % silt and clay, and organic carbon content for wormbed sediments collected around each of 3 worm species, and median grain size, % silt and clay, and organic carbon content for worm burrow lining sediments. Values are given as mean ± SD (n = 10) for each set of samples

Location	Median grain size (µm)	Silt and clay (%)	Organic C (%)
Balanoglossus aurantiacus wormbed	191 ± 2	0.9 ± 0.2	0.95 ± 0.08
Notomastus lobatus wormbed	167 ± 1	3.1 ± 1.4	2.16 ± 0.22
Branchyoasychus americana wormbed	163 ± 1	3.6 ± 1.5	2.04 ± 0.47
B. aurantiacus burrow	178 ± 3	0.7 ± 0.2	1.11 ± 0.12
N. lobatus burrow	162 ± 5	7.1 ± 2.3	3.90 ± 0.56
B. americana burrow	136 ± 10	7.9 ± 1.2	3.27 ± 0.31

has also been identified in the closely related species *Balanoglossus biminiensis* (Ashworth & Cormier 1967). *B. aurantiacus* is also sympatric with *Saccoglossus kowalewskyi* at this site, but is more abundant and more easily identified in the field. *B. aurantiacus* produces the same compound (2,6-dibromophenol) as the *S. kowalewskyi* used in previous studies (King 1986), while the *S. kowalewskyi* at this site produces bromopyrroles (Woodin et al. 1987, Fielman & Target 1995). Although a different genus and species than *S. kowalewskyi*, *B. aurantiacus* has the advantages of producing the same compound, producing similar burrow structure, and living in sediments similar to those of worms used in previous studies (King 1986, 1988). No volatile halogenated aromatic compounds were detected in *Branchyoasychus americana* (this study); King (1986) also found no volatile halogenated compounds in a closely related species, *Clymenella torquata*.

PLFA biomass

Burrow lining and surface and subsurface wormbed sediment microbial communities were examined using PLFA analysis. The mean quantities of the most abundant PLFA in the profiles for each sample set and sediment type are shown in Tables 2 to 4. Quantities are given as the mole percentage of the total amount of PLFA recovered as phospholipid fatty acid methyl esters. The transesterification process ensures recovery of only the membrane-bound PLFA, which have ester bonds linking the fatty acids to the glycerol head group. As a result, the analysis provides information primarily on viable microorganisms due to the rapid degradation of free bacterial phospholipids in marine sediments (White et al. 1979). A total of 60 fatty acids were identified in each profile, but many of these were present in very low quantities representing mole percentages less than 0.1% of the profile total. The PLFA listed in Tables 2 to 4 accounted for 93 to 97 mol% of the total PLFA present in each sample.

Mean PLFA biomass was generally highest in the surface sediments, intermediate in the burrow sediments, and lowest in the subsurface sediments (Tables 2 to 4, Fig. 1). Sample groups (burrow, surface, and subsurface) and worm species associated with the samples both had significant effects (2-way ANOVA: sediment type: MSE = 10.50, df = 2, F = 139.06, p ≤ 0.0001; worm species: MSE = 1.29, df = 2, F = 17.13, p ≤ 0.0001). Further analysis of PLFA biomass estimates using a Tukey test (Fig. 1) showed some exceptions. The *Notomastus lobatus* burrow and surface samples were homogeneous with respect to biomass, as were the *Balanoglossus aurantiacus* burrow and subsurface

samples. While significantly lower than the surface biomass in 2 out of 3 cases, PLFA biomass within burrows of all 3 worm species was substantial, more than 3 times the levels found in subsurface sediments. Total PLFA biomass was not significantly different (Tukey test) between the *Branchyoasychus americana* (no bromometabolites) and *N. lobatus* (bromophenols) burrow samples, nor between the *N. lobatus* and *B. aurantiacus* (dibromophenol) burrow samples. However, burrow microbial biomass significantly differed between *B. americana* and *B. aurantiacus*, the organisms inhabiting the least similar sediments and forming the most dissimilar burrow structures (Table 1, Fig. 1).

Infaunal burrows and ventilated sediments are well known to be sites of increased microbial biomass and activity (Aller 1978, 1983, Aller & Yingst 1978, Alongi 1985, Krantzberg 1985), most likely due to burrow properties and macrofaunal activities. Dobbs & Guckert (1988a) used PLFA analysis to show that the burrows of *Callianassa trilobata*, an infaunal shrimp, contained higher microbial biomass than surrounding sediments. The burrows used in this study, including the bromophenol-contaminated burrows, are no exception. Microbial biomass in the *Notomastus lobatus* burrow lining, which is exposed to >800 ng g^{-1} dry weight sediment of bromophenols (Lincoln et al. unpubl.), was as high as that observed in the surface sediments (Tables 2 & 3, Fig. 1). Similarly, Jensen et al. (1992) found burrow bacterial numbers twice as high as those in subsurface control sediments. These results suggest that elevated microbial biomass in infaunal burrows is more directly attributable to the physicochemical properties of the burrow and irrigation activities of the infaunal host organism (Boudreau & Marinelli 1994) than to the presence or absence of halo-aromatic secondary metabolites.

Microbial community composition

More detailed examination of the PLFA profiles revealed some striking differences between microbial communities in the 3 sample types (Figs. 2 & 3). Surface sediment microbial communities typically contained high levels of polyunsaturated phospholipid fatty acids (PUFA), including 20:4ω6 and 20:5ω3, while subsurface sediment communities had much lower levels of these PLFA. Subsurface sediments contained elevated levels of branched saturated fatty acids relative to the other sediment types (Figs. 2 & 3). PLFA structural groups recovered from burrow microbial communities were most often present at levels intermediate between those for surface and subsurface sediments.

Differences in microbial community structures were also observed among the different burrows examined

Table 2. Phospholipid fatty acid (PLFA) values presented as a mol% of total PLFA for burrow sediments from 3 marine worms. PLFA shown are the most abundant (> 0.1 mol%) fatty acids in the profiles. Sum SAFA: sum of all saturated PLFA; sum BRSAFA: sum of all terminally branched saturated PLFA; sum MUFA and Cy: sum of all monounsaturated and cyclopropyl PLFA; sum PUFA: sum of all polyunsaturated PLFA; sum meBRSAFA: sum of all methyl branched saturated PLFA; Cy/MUFA: ratio of cyclopropyl PLFA to the MUFA precursor; Trans/cis: ratio of trans to cis isomers of a given PLFA. Values presented are mean and standard deviation (n = 10) for each set of samples

| Sample type | Balanoglossus aurantiacus | | Notomastus lobatus | | Branchyoasychus americana | |
	Mean	SD	Mean	SD	Mean	SD
PLFA						
14:0	0.95	0.89	1.22	0.99	2.58	0.84
15:0	1.62	0.66	0.97	0.44	1.34	0.45
16:0	13.42	1.59	11.41	1.89	13.29	1.12
17:0	1.79	0.21	1.33	0.28	0.82	0.23
18:0	3.30	0.63	3.46	1.51	1.78	0.47
20:0	0.48	0.07	0.52	0.22	0.26	0.07
22:0	0.25	0.06	0.20	0.08	0.12	0.03
24:0	0.31	0.08	0.18	0.10	0.15	0.07
Sum SAFA	22.12		19.30		20.35	
i14:0	0.19	0.34	0.41	0.35	0.66	0.28
i15:0	2.18	1.08	2.57	1.42	3.98	1.01
a15:0	3.35	1.66	3.00	2.01	4.89	1.25
i16:0	1.10	0.25	1.14	0.51	1.34	0.29
i17:0	0.76	0.04	0.74	0.22	0.71	0.11
17:1ω8c/a17:0	3.53	0.24	2.25	0.57	2.12	0.43
Sum BRSAFA	11.45		8.02		14.02	
a16:0/16:1ω9c	0.59	0.12	0.89	0.33	1.03	0.20
16:1ω7c	10.92	2.15	13.94	5.05	16.77	1.97
16:1ω7t	0.37	0.08	0.42	0.15	1.06	0.18
16:1ω5c	1.33	0.25	1.57	0.52	2.13	0.67
16:1ω13t	0.37	0.09	0.14	0.07	0.22	0.12
17:1ω6c	1.03	0.21	0.72	0.24	0.67	0.14
cy17:0	0.51	0.15	0.45	0.24	0.75	0.21
18:1ω9c	3.25	0.47	2.85	0.60	1.98	0.43
18:1ω7c	12.16	1.84	13.67	3.39	20.20	7.04
cy19:0(ω7,8)	0.44	0.08	0.92	0.67	4.02	2.83
Sum MUFA and Cy	33.26		38.30		50.41	
16:2ω6/br15:0	0.49	0.14	0.31	0.14	0.33	0.12
poly17	0.38	0.13	0.12	0.06	0.18	0.08
18:3ω6	0.50	0.08	0.32	0.19	0.51	0.13
18:4ω3/12me17:0	0.68	0.26	0.46	0.24	0.59	0.12
18:2ω6	0.59	0.21	0.88	0.59	0.32	0.27
18:3ω3/br17:1	2.00	0.31	1.19	0.59	1.33	0.25
20:4ω6	6.44	1.87	4.26	1.35	1.77	1.16
20:5ω3	7.60	2.24	6.53	3.46	1.97	1.86
20:3ω6	0.41	0.13	0.26	0.09	0.14	0.08
20:4ω3	0.68	0.46	0.33	0.13	0.06	0.03
20:3ω3	0.85	0.34	2.59	1.78	0.55	0.70
22:5ω6	1.17	0.27	0.93	0.30	0.21	0.30
22:6ω3	2.41	0.75	2.75	1.48	0.72	0.85
22:4ω6	0.55	0.16	1.38	0.80	0.21	0.30
22:5ω3	0.68	0.18	1.41	1.65	0.24	0.43
poly22	2.56	2.07	1.33	2.15	0.03	0.03
Sum PUFA	29.11		26.28		9.58	
10me16:0	2.17	0.19	3.25	1.31	3.35	0.69
10,11me18:0	0.47	0.03	0.66	0.31	0.54	0.13
Sum meBRSAFA	2.97		4.30		4.09	
Sum of listed PLFA	94.86		93.92		95.91	
Total pmol g^{-1} dry wt	11 584	5 186	36 955	21 731	55 899	29 540
Cy/MUFA						
cy17:0/16:1ω7c	0.05	0.01	0.03	0.02	0.04	0.01
cy19:0/18:1ω7c	0.04	0.009	0.06	0.04	0.17	0.08
Trans/cis						
16:1ω7t/16:1ω7c	0.03	0.005	0.03	0.01	0.06	0.02
18:1ω7t/18:1ω7c	0.03	0.004	0.03	0.02	0.03	0.006

Table 3. PLFA values presented as a mol% of total PLFA for surface sediments near burrows of 3 marine worms. See Table 2 legend for details

| Sample type | *Balanoglossus aurantiacus* | | *Notomastus lobatus* | | *Branchyoasychus americana* | |
	Mean	SD	Mean	SD	Mean	SD
PLFA						
14:0	4.30	1.58	2.07	0.96	7.33	1.14
15:0	3.66	0.58	1.63	0.39	3.13	0.22
16:0	17.45	0.45	17.30	1.95	17.08	0.86
17:0	1.38	0.15	1.13	0.20	1.06	0.12
18:0	2.32	0.32	2.80	1.32	1.90	0.19
20:0	0.28	0.04	0.33	0.18	0.24	0.02
22:0	0.22	0.08	0.21	0.12	0.17	0.02
24:0	0.44	0.06	0.25	0.07	0.32	0.07
Sum SAFA	29.82		25.73		31.23	
i14:0	0.46	0.26	0.25	0.11	1.06	0.07
i15:0	1.95	0.37	1.15	0.35	2.54	0.16
a15:0	2.40	0.47	1.37	0.41	3.05	0.20
i16:0	0.74	0.06	0.67	0.15	0.80	0.04
i17:0	0.46	0.04	0.45	0.09	0.44	0.02
17:1ω8c/a17:0	3.06	0.24	1.93	0.36	2.18	0.14
Sum BRSAFA	9.09		5.80		10.07	
a16:0/16:1ω9c	0.74	0.07	0.75	0.16	0.95	0.05
16:1ω7c	15.07	0.93	14.43	2.50	16.20	1.20
16:1ω7t	0.23	0.07	0.26	0.05	0.35	0.03
16:1ω5c	1.23	0.12	1.04	0.16	1.27	0.06
16:1ω13t	1.17	0.12	0.96	0.20	1.16	0.13
17:1ω6c	0.76	0.12	0.60	0.11	0.71	0.10
cy17:0	0.03	0.07	0.23	0.07	0.21	0.03
18:1ω9c	2.52	0.24	3.61	0.39	2.60	0.23
18:1ω7c	7.79	0.91	9.35	1.19	7.55	0.56
cy19:0(ω7,8)	0.15	0.06	0.38	0.13	0.18	0.03
Sum MUFA and Cy	31.36		33.77		32.69	
16:2ω6/br15:0	1.04	0.18	0.71	0.21	0.99	0.28
poly17	1.07	0.23	0.55	0.15	0.58	0.08
18:3ω6	0.52	0.07	2.21	0.47	0.37	0.05
18:4ω3/12me17:0	1.39	0.15	nd	nd	1.28	0.11
18:2ω6	0.83	0.11	0.98	0.13	0.82	0.10
18:3ω3/br17:1	0.76	0.08	1.12	0.28	0.81	0.06
20:4ω6	3.31	0.49	3.51	0.47	2.67	0.44
20:5ω3	11.52	0.87	13.87	2.48	10.00	1.04
20:3ω6	0.20	0.08	0.30	0.04	0.20	0.04
20:4ω3	0.32	0.05	0.41	0.07	0.30	0.05
20:3ω3	0.18	0.09	0.97	0.65	0.24	0.07
22:5ω6	1.02	0.18	0.78	0.10	0.79	0.09
22:6ω3	4.02	0.45	5.01	1.37	3.81	0.55
22:4ω6	0.20	0.07	0.27	0.09	0.16	0.04
22:5ω3	0.58	0.12	0.66	0.25	0.46	0.12
poly22	0.01	0.04	0.05	0.05	0.03	0.05
Sum PUFA	27.81		32.29		24.13	
10me16:0	0.78	0.11	1.21	0.25	0.82	0.12
10.11me18:0	0.02	0.03	0.36	0.05	0.08	0.03
Sum meBRSAFA	1.25		1.59		1.20	
Sum of PLFA listed	96.58		96.13		96.91	
Total pmol g^{-1} dry wt	57 996	14 611	48 959	18 700	117 459	24 624
Cy/MUFA						
cy17:0/16:1ω7c	0.00	0.004	0.02	0.007	0.01	0.004
cy19:0/18:1ω7c	0.02	0.006	0.04	0.008	0.03	0.005
Trans/cis						
16:1ω7t/16:1ω7c	0.02	0.007	0.02	0.006	0.02	0.004
18:1ω7t/18:1ω7c	0.02	0.008	0.02	0.003	0.02	0.005

Table 4. PLFA values presented as a mol% of total PLFA for subsurface sediments near burrows of 3 marine worms. See Table 2 legend for details

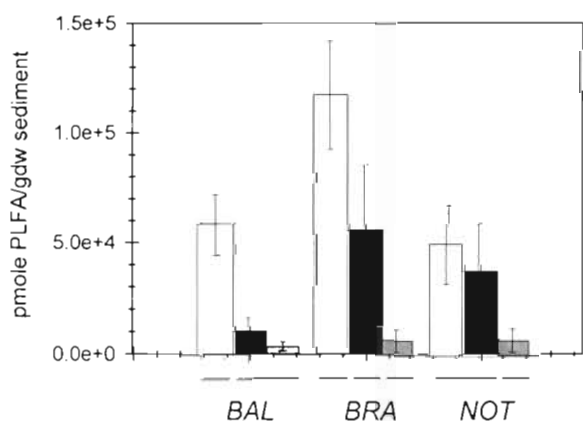| Sample type	Balanoglossus aurantiacus		Notomastus lobatus		Branchyoasychus americana	
	Mean	SD	Mean	SD	Mean	SD
PLFA						
14:0	1.61	1.78	1.48	0.96	2.36	0.69
15:0	2.09	1.28	1.52	0.69	1.58	0.46
16:0	14.86	6.26	14.43	2.69	14.36	1 69
17:0	1.83	0.76	1.64	0.30	1.30	0.22
18:0	3.12	1.36	3.54	1.46	2.95	0.63
20:0	0.68	0.22	1.18	0.57	1.10	0.42
22:0	0.36	0.11	0.42	0.27	0.49	0.18
24:0	0.40	0.15	0.36	0.21	0.44	0.15
Sum SAFA	24.95		24.58		24.58	
i14:0	0.69	0.47	0.55	0.47	1.02	0.47
i15:0	5.02	1.14	4.04	1.56	6.40	1.54
a15:0	8.07	1.76	6.23	2.62	10.86	2.47
i16:0	2.38	0.55	2.55	0.77	4.36	1.14
i17:0	1.25	0.36	1.38	0.13	1.61	0.23
17:1ω8c/a17:0	4.53	1.51	3.98	0.65	4.59	0.59
Sum BRSAFA	22.53		19.47		29.85	
a16:0/16:1ω9c	0.65	0.28	0.66	0.28	0.70	0.07
16:1ω7c	8.20	5.21	8.15	2.72	5.97	2.83
16:1ω7t	0.62	0.17	0.65	0.14	0.69	0.14
16:1ω5c	1.65	0.57	1.52	0.25	1.65	0.21
16:1ω13t	0.16	0.35	0.20	0.11	0.10	0.10
17:1ω6c	0.93	0.42	0.79	0.24	0.53	0.25
cy17:0	0.81	0.25	0.69	0.12	0.85	0.11
18:1ω9c	3.39	1.35	3.30	0.53	2.11	0.65
18:1ω7c	10.26	4.98	10.17	1.79	7.97	1.89
cy19:0(ω7,8)	0.70	0.21	0.97	0.31	0.87	0.18
Sum MUFA and Cy	29.71		30.24		23.45	
16:2ω6/br15:0	0.46	0.29	0.47	0.11	0.56	0.10
poly17	0.29	0.32	0.12	0.05	0.09	0.09
18:3ω6	0.68	0.23	0.93	0.16	0.78	0.14
18:4ω3/12me17:0	0.38	0.42	0.18	0.29	0.34	0.16
18:2ω6	0.39	0.28	0.75	0.25	0.34	0.22
18:3ω3/br17:1	4.33	1.22	3.51	1.41	5.93	1.68
20:4ω6	2.74	2.82	2.90	1.33	1.16	0.90
20:5ω3	1.98	3.93	1.90	1.58	1.21	1.18
20:3ω6	0.27	0.18	0.64	1.00	0.12	0.08
20:4ω3	0.26	0.41	0.23	0.12	0.26	0.55
20:3ω3	0.41	0.44	0.99	0.75	0.52	0.19
22:5ω6	0.70	0.48	0.74	0.30	0.37	0.24
22:6ω3	0.74	1.32	1.23	1.57	0.53	0.69
22:4ω6	0.31	0.24	0.61	0.67	0.20	0.29
22:5ω3	0.18	0.29	0.29	0.37	0.16	0.25
poly22	0.06	1.89	0.20	0.38	0.12	0.09
Sum PUFA	15.16		16.67		13.02	
10me16:0	4.33	1.25	5.13	0.87	5.11	0.79
10.11me18:0	1.09	0.37	1.53	0.67	1.48	0.38
Sum meBRSAFA	6.07		7.58		7.77	
Sum of PLFA listed	93.85		92.77		94.13	
Total pmol g^{-1} dry wt	3 411	2 091	6 524	5 203	6 489	4 951
Cy/MUFA						
cy17:0/16:1ω7c	0.11	0.04	0.09	0.04	0.16	0.07
cy19:0/18:1ω7c	0.07	0.010	0.09	0.02	0.11	0.03
Trans/cis						
16:1ω7t/16:1ω7c	0.08	0.010	0.09	0.03	0.12	0.03
18:1ω7t/18:1ω7c	0.05	0.007	0.06	0.02	0.05	0.010

Fig. 1 Microbial biomass, as measured by total membrane PLFA for surface (□), worm burrow lining (■), and subsurface (▨) sediment samples for 3 worm species. *BAL: Balanoglossus aurantiacus; NOT: Notomastus lobatus; BRA: Branchyoasychus americana.* Values represent mean ± SD for 10 samples. Lines under the x-axis are results of the Tukey test at a significance level of α = 0.01. Sample types connected by a common line are not significantly different. ANOVA-*F* significance was p ≤ 0.0001 for all groups, error df = 27

(Figs. 2 & 3). Only normal saturated PLFA did not differ significantly among the burrow lining samples. The significant difference between terminally branched saturated PLFA levels in *Notomastus lobatus* and *Branchyoasychus americana* burrows was largely due to
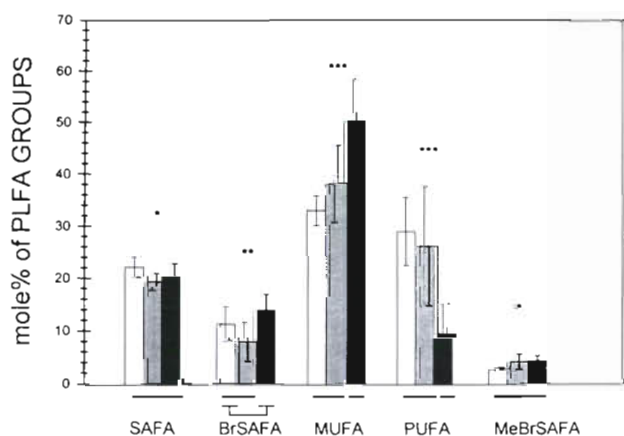
Fig. 2. Microbial community structure as represented by PLFA groups for burrow sediment sample sets collected from three different species of marine worms, *Notomastus lobatus* (□), *Balanoglossus aurantiacus* (▨), and *Branchyoasychus americana* (■). SAFA: saturated phospholipid fatty acids; BrSAFA: branched saturated PLFA; MUFA: monounsaturated PLFA; PUFA: polyunsaturated PLFA; MeBrSAFA: methyl branched saturated PLFA. See Fig. 1 legend for Tukey test description. ANOVA-*F* significance as follows: no star: no significant difference among groups, •p ≤ 0.01, ••p ≤ 0.001, •••p ≤ 0.0001. Error df = 27
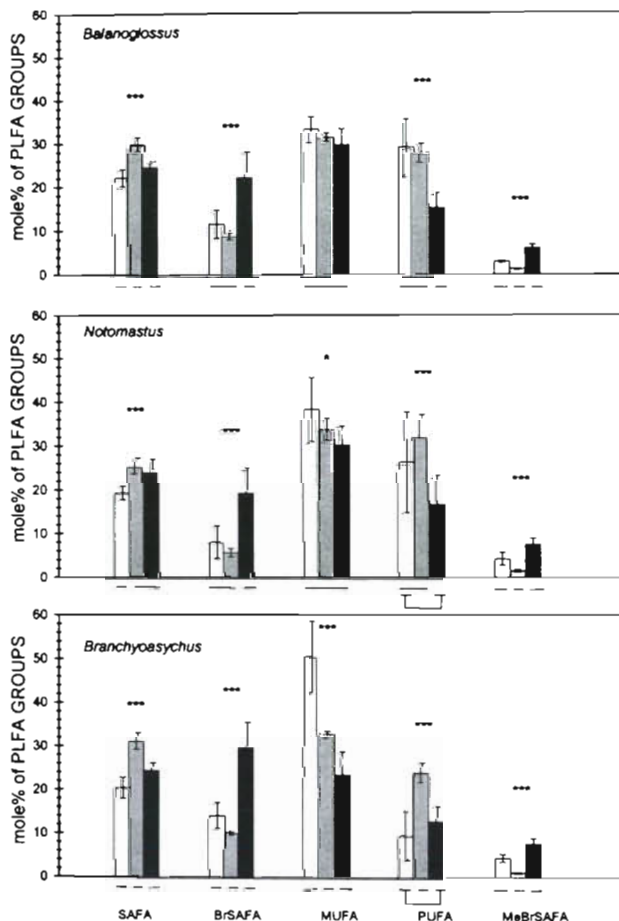
Fig. 3. Microbial community structure as represented by PLFA groups for sediment sample sets; burrow (□), surface (▨), and subsurface (■) sediments collected around *Balanoglossus aurantiacus, Notomastus lobatus,* and *Branchyoasychus americana.* See Fig. 1 & 2 legends for Tukey test description, identification of PLFA groups, and ANOVA-*F* significance. Error df = 27

higher amounts of i,a15:0 occurring in the *B. americana* burrows. Monounsaturated and cyclopropyl PLFA in *B. americana* burrows were significantly different from both *Balanoglossus aurantiacus* and *N. lobatus* burrow sediments. This was due to increased mole percentages of 16:1ω7c, 18:1ω7c, and cy19:0(ω7,8) in the *B. americana* burrow sediments. PUFA were most abundant in (and not significantly different between) the *B. aurantiacus* and *N. lobatus* burrow sediments. PUFA levels in *B. americana* burrow sediments were significantly lower than in either of the other burrow types, due mainly to decreased levels of 20:4ω6 and 20:5ω3. These PUFA are abundant in eucaryotic organisms, with diatoms being the primary contributors in intertidal marine sediments (Erwin 1973, Orcutt & Patterson 1975, Volkman et al. 1980, White 1983, Nichols et al. 1986, Smith et al. 1986). Methyl branched saturated

fatty acids were significantly different among burrow sediments from the 3 species by ANOVA (p ≤ 0.01), but the Tukey test showed no significant differences among the 3 sample groups for these fatty acids.

Multivariate cluster analysis

Due to the size and complexity of the sample set, multivariate cluster analysis was used to relate samples or species based on the total PLFA profiles. Principal components analysis (PCA) was used to identify the variables (individual PLFA) which accounted for the majority of the variance among the PLFA profiles (Table 5). As was seen from the biomass and PLFA structural group evaluations, clusters constructed using PLFA mole percentage values for all 9 of the sample categories (3 worms × 3 sediment types) showed that PLFA from the 3 types of sediments (surface, burrow, subsurface) were distinct (Fig. 4). The fidelity or accuracy of the clusters of sample types for the complete sample set was very high. The burrow cluster included 22 of the 30 burrow samples (similarity 0.51), and 1 subsurface sediment sample. This subsurface sample was characterized by elevated levels of 16:1ω7c, 20:5ω3 and 20:3ω3 relative to other subsurface samples in the set and may have been affected by the strong sediment mixing processes at this site (Grant 1983). Two other burrow samples, *Branchyoasychus americana* B6 and B7, clustered by themselves and contained very high mole percentages of 18:1ω7c. This could be due to increased levels of either bacterial or microeucaryotic community members. The surface sediment cluster included 29 of the 30 surface sediment samples (similarity 0.67) and no members from other groups. The subsurface sediment cluster included 29 of 30 subsurface sediment samples (similarity 0.51). Two burrow samples, 1 *Notomastus lobatus* and 1 *Balanoglossus aurantiacus*, were also included in this subsurface cluster. These 2 burrow samples contained higher mole percentages of a15:0 and lower mole percentages of 18:1ω7c, 20:4ω6, 20:5ω3 and 22:6ω3, consistent with values seen in the other subsurface samples. It is likely that these samples included some subsurface sediment or were taken from vacant burrows with little or no burrow water circulation. In addition most sample sets collected for each worm species (i.e. burrow, surface and subsurface sediments for a species) also segregated into the same distinct clusters, though the fidelity of these clusters was lower, with more outliers and mixed

clusters (Figs. 5 to 7). This was likely due to the decreased statistical power resulting from separate analysis of the much smaller data sets from each species relative to the complete catalog of PLFA profiles from all species and sediment types. Most clusters contained 5 to 10 members, with some outliers. For example, *N. lobatus* samples B2 and B9 were outliers from the primary cluster of burrow samples from this set (Fig. 5). Similarly 3 *B. aurantiacus* subsurface sediment samples (D1, D3 and D4) were outliers from the primary cluster (Fig. 6).

Principal components analysis

PCA was used to identify a variety of PLFA which account for a significant portion of the variance found among the profiles (Table 5). PCA loadings for PLFA indicate that monounsaturated fatty acids (MUFA), PUFA, branched saturated fatty acids, and 10me16:0 all represent important components of the sediment microbial communities. PCA loadings in conjunction with analysis of the mean PLFA values in the different sample sets were used to identify which PLFA explained the greatest level of variability among the different types of sediments. These PLFA included a15:0, i15:0, 10me16:0, br17:1, and 17:1ω8c for subsurface sediments, 20:5ω3 and 16:1ω7c for burrow and surface sediments, 18:1ω7c, 20:5ω3 and 20:4ω6 for burrow sediments, and 16:0 and 14:0 for surface sediments. Many of these fatty acids were highly abundant in the samples (Tables 2 to 4). This information also forms the basis for analysis of indicator PLFA in the different sediment types.

Table 5. PLFA and their coefficients of loading from principal components analysis for principal component 1 (PC 1), principal component 2 (PC 2), and principal component 3 (PC 3) which together accounted for 89% of the variability within the data set. These were the 10 most variable components after analysis of all burrow, surface and subsurface sediment sample data from the *Balanoglossus aurantiacus*, *Notomastus lobatus*, and *Branchyoasychus americana* sample sets

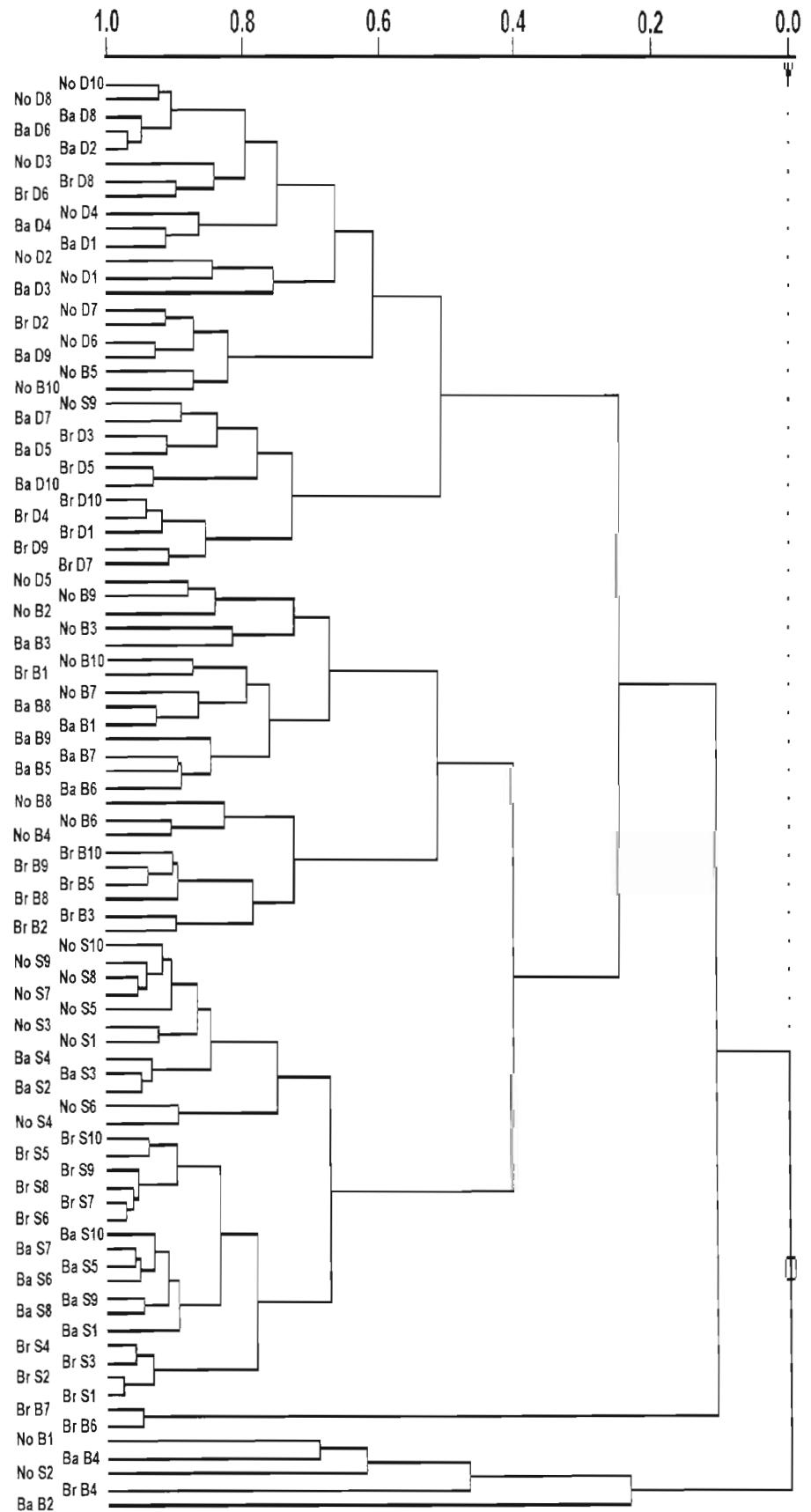| PC 1 50% of variability		PC 2 34% of variability		PC 3 5% of variability	
20:5ω3	0.6066	18:1ω7c	0.8342	16:1ω7c	0.5941
a15:0	−0.4053	16:1ω7c	0.3016	16:0	0.3487
16:1ω7c	0.3993	20:5ω3	−0.2348	20:4ω6	−0.3109
22:6ω3	0.2379	cy19:0	0.1936	14:0	0.3021
10me16:0	−0.2248	a15:0	−0.1665	20:3ω3	−0.2290
i15:0	−0.2242	16:0	−0.1599	18:0	−0.2150
18:3ω3	−0.2234	14:0	−0.1260	20:5ω3	−0.2033
i16:0	−0.1532	18:3ω3	−0.1062	a15:0	0.1973
20:4ω6	−0.1280	15:0	−0.0810	22:6ω3	−0.1769
16:0	−0.1192	22:6ω3	−0.0807	i15:0	0.1627

Fig. 4. Dendrogram from multivariate cluster analysis, farthest neighbor complete linkage, of PLFA profiles from 90 samples collected around *Notomastus lobatus* (No), *Balanoglossus aurantiacus* (Ba), and *Branchyoasychus americana* (Br) burrows, including 10 burrow sediment samples (B), 10 subsurface sediment samples (D), and 10 surface sediment samples (S) for each worm species. Line distances between clusters and indices indicate degree of similarity between clusters. An index of 1.000 indicates identical samples
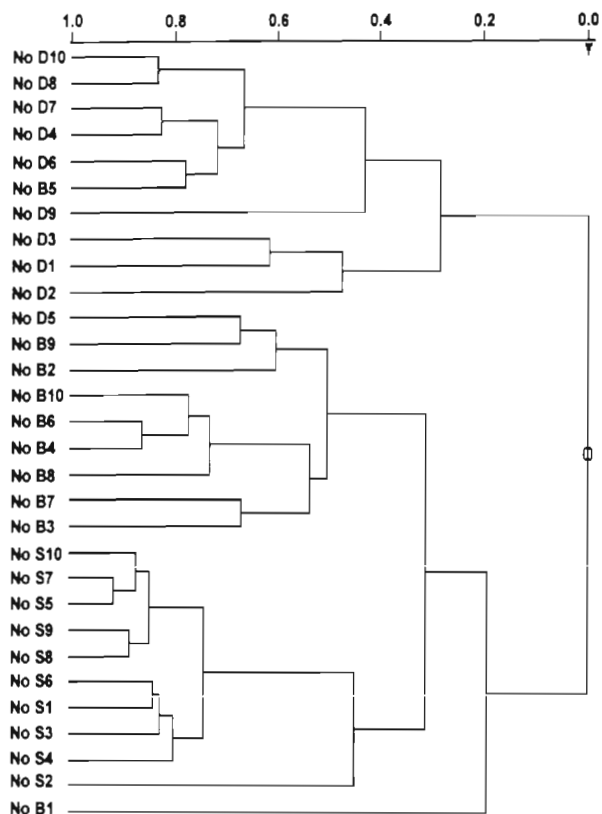
Fig. 5. Dendrogram from multivariate cluster analysis, farthest neighbor complete linkage, of PLFA profiles from a 30 sample set collected around *Notomastus lobatus* burrows. See Fig. 4 legend for details

Biomarker PLFA

Signature biomarker PLFA, which can be highly correlated with certain groups of microorganisms, were identified in all samples (Fig. 8). Several fatty acids have previously been used as indicators of bacterial biomass in marine sediments. Two particularly abundant PLFA in bacteria are 18:1ω7c and 16:1ω7c (Perry et al. 1979, Gillan et al. 1983, Parkes & Taylor 1983, Guckert et al. 1985, Currie & Johns 1988), although both of these MUFA are also found in eucaryotes (Johns et al. 1979, Gillan et al. 1981, Nichols et al. 1986). Total MUFA and cyclopropyl fatty acids in burrow samples ranged from 33 to 50 mol% of the total and PUFA ranged from 9 to 29 mol%, with the highest ratio of MUFA:PUFA found in the *Branchioasychus americana* burrow sediments. The MUFA 18:1ω7c and 16:1ω7c were abundant within the worm burrows, together accounting for 23 to 37% of the total PLFA abundance. They were also abundant in the surface (mean = 23% of total PLFA) and subsurface sediments (mean = 17% of total PLFA). Although we cannot dis-

count that these 2 PLFA are found in microeucaryotic cell membranes, they are also terminal points in biosynthetic pathways utilized in all Gram-negative bacteria. It is expected that the presence of these PLFA is also due in large part to bacterial PLFA synthesis, providing evidence that bacteria contribute a significant component of microbial biomass in all 3 sediment types (Fig. 8D)

PLFA indicative of sulfate reducers were identified in each of the sediment types examined (Fig. 8A). The abundance of 10me16:0, a major membrane component of the sulfate reducer *Desulfobacter* sp. (Kohring et al. 1994), as well as some other obligately anaerobic bacteria (Taylor & Parkes 1983, Edlund et al. 1985, Dowling et al. 1986, 1988, Vainshtein et al. 1992), showed the same trends in all of the sample sets. Mole percentages increased in the order: surface sediments < burrow sediments < subsurface sediments (Fig. 8A). This branched saturated fatty acid accounted for 2.2 to 3.5% of the total PLFA in the burrow sediments. Other branched saturated PLFA are also indicative of anaerobic and sulfate reducing bacteria in marine sediments. Trends in the mole percentages for this group of
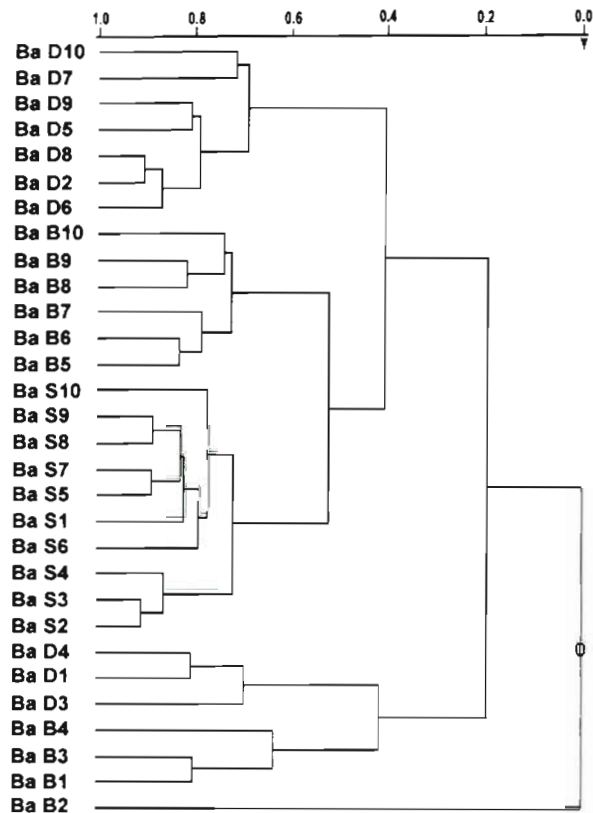

Fig. 6. Dendrogram from multivariate cluster analysis, farthest neighbor complete linkage, of PLFA profiles from a 30 sample set collected around *Balanoglossus aurantiacus* burrows. See Fig. 4 legend for details
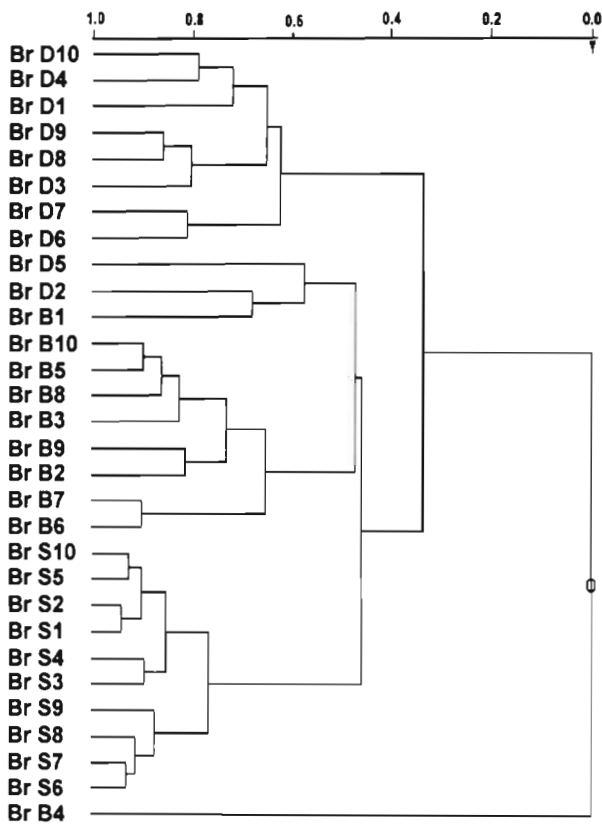
Fig. 7. Dendrogram from multivariate cluster analysis, farthest neighbor complete linkage, of PLFA profiles from a 30 sample set collected around *Branchyoasychus americana* burrow. See Fig. 4 legend for details

PLFA are the same as that of 10me16:0 (Fig. 8C). The high levels of PLFA indicative of sulfate reducers found in the subsurface sediments are consistent with sulfate reducer ecology and with other studies of marine sediments (Perry et al. 1979, Jørgensen 1980, Parkes & Taylor 1983, Findlay et al. 1985, 1990, Dobbs & Guckert 1988a, b). The presence of sulfate reducing bacteria in surface sediments can be explained by the presence of reduced microniches, such as fecal pellets (Jørgensen 1977), in oxidized marine sediments. Worm burrow lining sediments are organically enriched, irrigated with overlying seawater containing substantial levels of sulfate, and contain anaerobic microzones, making them an excellent niche for sulfate reducing bacteria (Aller 1978). Interestingly, the tightly consolidated and apparently poorly irrigated tubes of *Branchioasychus americana* contained high levels of branched saturated PLFA, indicative of sulfate reducers and other anaerobic bacteria.

PUFA with chain lengths of C19 and longer are considered biomarkers for eucaryotic organisms, particularly diatoms in estuarine sediments (Erwin 1973, Orcutt & Patterson 1975, Volkman & Johns 1977, Volkman et al. 1980, Gillan et al. 1981, Nichols et al. 1986, Smith et al. 1986, Vestal & White 1989). Eucaryotic biomass, indicated by the abundance of the PLFA 16:2ω6, 20:4ω6, 20:5ω3, 20:3ω3, 22:6ω3 and 22:5ω3, showed the same trends in all sample sets, decreasing in the order surface ≥ burrow ≥ subsurface (Fig. 8B). This distribution of PUFA is consistent with

Fig. 8. Distribution of specific microbial groups around burrows of 3 species of infaunal worms. *Notomastus lobatus* (□), *Balanoglossus aurantiacus* (▨), *Branchyoasychus americana* (■). (A) Sulfate reducing and other anaerobic bacteria based on 10me16:0, a signature lipid biomarker for *Desulfobacter* sp. (B) Eucaryotic organisms (primarily diatoms) based on 16:2ω6, 20:4ω6, 20:5ω3, 20:3ω3, 22:6ω3 and 22:5ω3. (C) Gram-positive bacteria based on i15:0, a15:0, i16:0, and i17:0. (D) Gram-negative bacteria based on monounsaturated PLFA (excluding 18:1ω9c, the eucaryotic precursor to polyunsaturated PLFA) and cyclopropyl PLFA
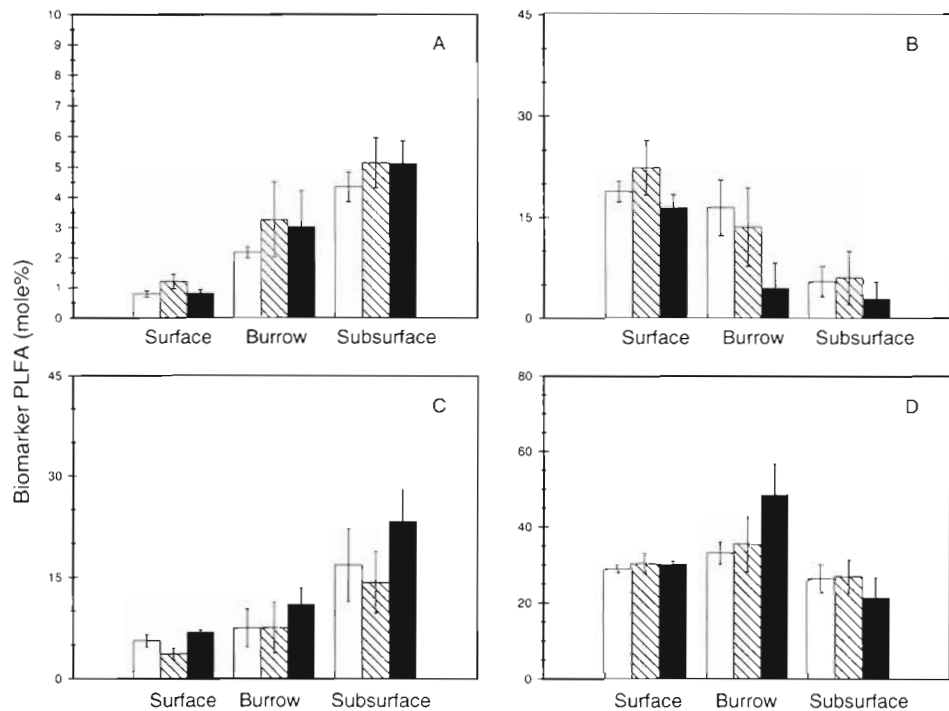
the known ecology of diatoms. Previous studies have found extensive and highly active microalgal communities at these and similar sites (Steward et al. 1992, Pinckney & Zingmark 1993). The presence of low levels of diatom-indicator PLFA in subsurface sediments is consistent with reports of viable diatoms in anoxic, aphotic subsurface marine sediments (Wasmund 1989) and may represent diatoms buried by sediment mixing processes. As was the case for bacterial biomass, substantial viable eucaryotic biomass, as represented by PUFA, was found within the worm burrows. Diatoms and/or other eucaryotes could either be transported into the burrows by the feeding and irrigation activities of the worms, or simply fall or be washed into the burrow opening from surface sediments. The lower level of diatom PLFA biomarkers in the burrows of *B. americana* (Fig. 8B) could be explained by the elevated tube opening, which represents a physical barrier that could decrease surface sediment (and diatom) transport into the worm burrow.

Microbial physiological status

Previous investigators have used the ratios of *trans* to *cis* isomers of specific MUFA as indicators of bacteria growing under physiologically stressful conditions (Guckert et al. 1986, Findlay et al. 1990). The highest ratios of *trans* to *cis* fatty acids were found in subsurface sediments, although mean values there were only slightly higher than 0.1, the lower limit for ratios typically interpreted to indicate stress (Tables 2 to 4). All burrow *trans/cis* ratios were lower than 0.1, similar to those in the surface sediments. The higher ratios of *trans* to *cis* fatty acids seen in the subsurface sediments (Table 3) are consistent with data from other investigators (Guckert et al. 1986, Findlay et al. 1990) and are thought to reflect a common physiological adaptation to growth under oligotrophic conditions. High (>0.1) *trans* to *cis* ratios for the fatty acids $16:1\omega7t/16:1\omega7c$ and $18:1\omega7t/18:1\omega7c$ and high levels of cy17:0 and cy19:0 were also seen in anaerobic cultures enriched from marine sediment (Guckert et al. 1985). Much lower ratios were observed in the burrows, possibly reflecting the higher degree of organic enrichment there (Table 1). The presence of cyclopropyl fatty acids and the ratio of cyclopropyl to precursor MUFA (e.g cy17:0/16:1ω7c) have also been identified as indicators for physiological changes related to stress or anoxia (Guckert et al. 1985). The distribution of cyclopropyl fatty acids in our sediment samples is consistent with anoxia driven physiological changes and also refutes physiologically stressful conditions within the burrows. Cyclopropyl fatty

acids and their associated ratios were typically highest in subsurface sediment samples, intermediate in burrow sediments and lowest in surface sediments (Tables 2 to 4). *Branchyoasychus americana* burrow sediments differed, having a high ratio of cy19:0/18:1ω7c. This may be explained by the apparent stagnation of *B. americana* burrow water and the resulting anoxia of the tube sediments. The physiological status of the worm burrow microbial communities indicates an environment congenial to growth of bacteria, even when bromoaromatic exudates are present. Based on high levels of microbial biomass, substantial diversity (as reflected by the plethora of fatty acids identified in these samples), low stress indices, and the consistency of our results from burrows constructed by 3 different worms, the burrow environment appears to be highly conducive to bacterial growth.

Concluding remarks

Although the microbial communities in burrows of each of the worm species we examined have certain unique characteristics, these communities are still more similar to each other than to those in nearby surface and subsurface sediments. Undoubtedly the burrow microenvironment established by any infaunal species has properties resulting in greater or lesser representation of some bacterial groups. Which burrow features or host characteristics are most important in establishing the composition of the burrow lining microbial community are presently unknown. Production of bromometabolites by worms has been hypothesized to inhibit growth of aerobic bacteria in the burrows (Ashworth & Cormier 1967, Sheikh & Djerassi 1975, King 1986, Goerke & Weber 1991), obviously altering the structure of the burrow microbial community. King (1988) reported such inhibition of aerobic bacteria in burrow sediments of the dibromophenol producer *Saccoglossus kowalewskyi*. This conclusion was based on low rates of glucose oxidation in contaminated sediments and absence of dibromophenol degradation by mixed aerobic cultures enriched from dibromophenol-contaminated sediments. However, our data, which reflect *in situ* biomass and community structure of undisturbed burrow lining biofilms, show no significant impact of biogenic bromophenols on microbial biomass, activities, or community structure. Surface and subsurface wormed sediment communities were similarly unaffected (Steward et al. 1992). Determining which burrow and macrofaunal host features control the formation and composition of the burrow lining biofilm community will require further investigation.

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